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Sanchored PCR: PCR with cDNA coupled to a solid phase

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We describe the use of oligonucleotides covalently coupled via an aminolink to a solid support to facilitate PCR experiments.

Aminolink 2 (Applied Biosystems) containing oligonucleotides were synthesized according to the manufacturer's protocol. They were coupled to N-hydroxysuccinimide 4% cross-linked agarose (Sigma). For coupling the agarose was first washed twice with water. Then 1/10 vol 2 M $Na_2CO_3/NaHCO_3$ buffer pH 9.5 was added to the oligonucleotide solution (100 nmol/500 µl) and a tenfold molar excess of N-hydroxysuccinimide coupled on agarose was resuspended in this solution. The suspension was placed at room temperature overnight and the reaction was stopped by adding 1/10 vol 3.3 M glycine solution. After two hours, the suspension was washed two times with water and resuspended in 100 µl of water. The oligonucleotides on the beads were stored at 4°C in an aqueous suspension.

Reverse transcription was performed in 10 µl reactions containing 2 µl bead suspension according to the manufacturer's protocol (BRL). The reverse transcription was performed in the same PCR tube that was also used for amplification as we found it hard to transfer small amounts of agarose. The first strand of cDNA generated this way is covalently linked to the solid support. The beads were pelleted, the supernatant removed, vortexed in 500 µl of water and repelleted. Using radioactive nucleotides we saw that >98% of the unincorporated nucleotides were removed after this step. The PCR mix is applied directly to the bead pellet. The cDNA coupled to the beads can be reused several times in PCR experiments, making it possible to optimise conditions like buffer, $MgCl_2$, enzyme concentration or profile conditions (Fig. 1A and 1C). The primer SS020 (sequence of the primer SS020 Aminolink 2 GCCTTCGAATTCAGCACCT$_{12}$ that we used to prime mRNA contained an adaptor primer that could be used in RACE (1) PCR experiments, indicating that sequences next to the aminolink and the bead are accessible for primers in a PCR reaction (Fig. 1D).

We were also able to use oligonucleotides coupled to agarose beads for affinity isolation of RNA as described in (2). Instead of eluting the RNA from the oligo (dT) it is directly reverse transcribed and can be reused several times. Furthermore, using one primer containing an aminolink, either strand of the PCR product can be coupled to a solid support following the same protocol that we used for oligonucleotides. This way long, specific sequences can be coupled to a solid matrix that might be useful in affinity isolation of nucleic acids.

The major advantage of solid anchored PCR (Sanchored PCR) is that by binding the cDNA to a solid phase, the cDNA is concentrated in a small volume and can be easily manipulated in different buffer systems. A portion of cDNA attached to a solid phase has the same information as a cDNA library. By using different coupled oligonucleotides as primers, specific 'libraries' can be constructed using the coupled oligonucleotides as an affinity matrix in RNA isolation.

Table 1: Reaction conditions for reprobing
1: annealing temperature; 2: annealing time in minutes (') or seconds (")

lane	oligo.	1	2	$MgCl_2$ mM	Gene	Ref. size, bp
A	SRS015/SRS016	60°C	1'	1.5	NILE	(4) 238
B	SS003/SS004	55°C	1'	2.0	Clathrin LCB	(5) 152
C	SRS015/SRS016	60°C	10'	2.0	NILE	(4) 238
D	SS015/SS021	55°C	1'	1.5	anchor primer/ID part	(6) various
E	SS032/SS033	55°C	1'	1.5	Clathrin LCA	(5) 256

SRS015: CTCGAGAAGCTTGCCGATGAAAGAGCCATCCTCATTGAACTG
SRS016: CTCGAGAAGCTTGCCGATGAAAGAGCCATCCTCATTGAACTG
SS003: TGCCTCGAAGGTGACCGAACAG SS004: GGTCTCCTCCTTGG-ATTCTTTC SS015: GCCTTCGAATTCAGCACC SS021: GGGGTTGGGG-ATTTAGC SS032: TGGTACGCAAGGCAGGATGAGC SS033: AGATCG-GAGACGTAGTGTTTCCA

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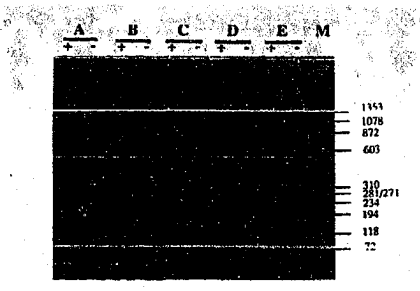

Figure 1. Successive probing of cDNA attached to agarose beads. 1 µg total liver RNA was reverse transcribed with SS020 attached to agarose beads and probed with the PCR mixes and reaction conditions specified in table 1. After each PCR reaction the old reaction mix was removed, the beads were washed one time with water, repelleted and reused. Denaturation was for 30 sec at 94°C, extension was for 2 min at 72°C, 30 cycles were used. The buffer contained 16.6 mM $(NH_4)_2SO_4$, 67 mM Tris-HCl, pH 8.8 and the $MgCl_2$ concentration and primers as indicated in table 1. The accuracy of the machines was controlled with an external thermocouple (3). Note that the 700 bp artifact band in lane A disappears in lane C due to different PCR conditions indicating that the washing effectively removes products of prior reactions. The lanes indicated '–' were performed using beads and RNA without reverse transcription. In lane D several different products were expected, as one of the primers was part of a repetitive ID element (6). M: phiX174 HaeIII marker.